BIOLOGIA PLANTARUM 36 (1): 155-158; 1994

BRIEF COMMUNICATION

Purification of the major late M_r 89 000 heat-shock protein from spring barley seedlings

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Abstract

A fast and efficient method for the isolation of major late heat-shock protein (HSP89) from the seedlings of spring barley (*Hordeum vulgare* L. cv. Fatran) incubated at 40 °C for 15 h was described.

Although the heat-shock response was first demonstrated in *Drosophila melanogaster* (Ritossa 1962), further analysis has shown that all living organisms, practically from bacteria to man, produce heat-shock proteins (HSP) in response to heat and a variety of other stresses (Lindquist 1986). In contrast to animals, in plants the production of a complex group of 20 - 30 LM_r HSP was observed (Key *et al.* 1985). Studies on HM_r HSP have been carried out in plants very rarely. Based on the results of Heuss-LaRosa *et al.* (1987) that the HM_r (*i.e.* 70 000 and 80 000) HSP might be good candidates for heat tolerant proteins, in our work we have focused on this group of heat-shock induced proteins. Due to its massive accumulation, HSP89 was the protein of interest.

Barley seeds (*Hordeum vulgare* L. cv. Fatran) were sterilized and allowed to germinate in the dark for 72 h at 26 ± 1 °C. 200 g of 3-d-old seedlings (with endosperms removed) were transferred to the incubation buffer (1% sucrose in 1 mM K-phosphate buffer, pH 6.0) for 15 h at 40 °C. After incubation seedlings were ground under liquid N_2 and resuspended in 50 cm^3 of extraction buffer TNESMEG

Received 8 June 1993, *accepted* 17 July 1993.
Abbreviations: HM_r - high relative molecular mass; HSP - heat-shock proteins; LM_r - low relative molecular mass.
Acknowledgements: I am grateful to Dr. František Supek (Dept. of Biochemistry, Comenius University, Bratislava) for his facility and helpful discussions during the work, and to Mrs. Judita Blahutiaková for her skilful photographical assistance.

(10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 0.5 mM EDTA, 1 % sucrose, 2 % β-mercaptoethanol and 10 % glycerol) and extracted for 30 min at 0 to 4 °C. The purification method comprises a three-step sequential column chromatography involving anion-exchange, affinity, and gel filtration matrices. Polyacrylamide gel electrophoresis (PAGE), isoelectric focusing (IEF) and protein concentration determination were performed basically according to procedures described in Repka and Slováková (1993).

The data presented in this paper extend and confirm our previous findings (Repka 1990), that 3-d-old spring barley seedlings respond to prolonged heat-shock (15 h at 40 °C) by synthesizing a new set of proteins (at least 9 intensive bands as well as some corresponding to 26 °C proteins). Among these late HSP, three most prominent protein bands (M_r 89 000, 44 000 and 33 000) were induced (Fig.1). The similar pattern has independently been observed with the aid of autoradiography by Necchi *et al.* (1987).

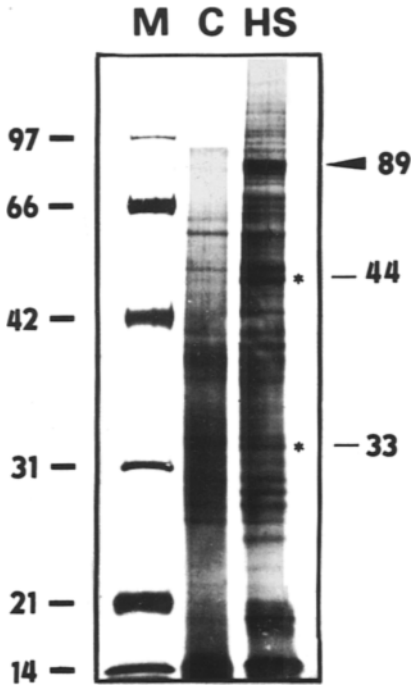

Fig. 1. Comparative 10 % SDS-PAGE slab gel electrophoresis of proteins extracted from control (C, 26 °C) and heat-shocked (HS, 40 °C) seedlings incubated for 15 h. Lane M contains a mixture of standard proteins.

A variety of methods used in the isolation of HSP from different sources were combined in a single procedure for the purification of the HSP89 from barley (Repka 1990). HSP89 was first recovered in the 0.15M NaCl eluate from the *DEAE - Cellulose* column and represented more than 30 % of the total protein content with an 88 % recovery in this single step (Table 1). HSP89 enriched fractions were then

applied to a *Blue Sepharose CL-4B* affinity column. Although the protein eluted as an asymetrical peak, the purity of HSP89 increased greatly in this process (Table 1). The final purification of the HSP89 was performed by gel filtration on *Sephadex G-75*. A single protein band was observed after *Coomassie Blue* staining. Even if the HSP89 was purified of only approximately 36-fold with an overall recovery of 70 %, the yield was satisfactory high; *ca.* 2 mg HSP89 was obtained using 200 g of stressed seedlings.

Table 1. Purification scheme for HSP89 from spring barley seedlings.

Step	Total proteins [mg]	HSP89* [mg]**	Purification [-fold]	Yield [%]
Crude extract	112.0	2.5	1.0	100
40 % $(NH_4)_2SO_4$	91.4	2.4	1.2	96
DEAE-Cellulose	7.3	2.2	14	88
Blue Sepharose CL-4B	2.8	2.0	32	80
Sephadex G-75	2.2	1.7	36	70

* - values represent HSP89 protein as calculated from A_{280} measurements of the final purification pool; ** - each value is the average of three separate experiments consistent with ± 0.05 mg cm^{-3}.

In preliminary characterization of the purified HSP89 we also had shown that it has an acidic isoelectric point (at about 5.2). The ultraviolet absorbance spectrum gave no evidence suggesting that the purified HSP89 contained any attached nucleotides. It is also interesting that similar result has been observed with purified avian heat-shock protein HSP90 (Iannotti *et al.*1988).

In conclusion, the availability of a rapid and reproducible method with high yields should aid in the preparation of specific antibody as well as in the structural analysis of this protein and thereby help in understanding its possible role in the process of thermoadaptation.

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